

Xylella fastidiosa invasion of new countries in Europe, the Middle East and North Africa: Ranking the potential exposure scenarios

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Abstract

After the recent high-impact European outbreaks of *Xylella fastidiosa* (*Xf*), a xylem-limited plant pathogenic bacterium native to the Americas, this research aims to rank the risks of potential entry, establishment and spread of *Xf* in new countries across Europe, the Middle East and North Africa. A novel risk-ranking technique is developed, based on combining entry risk drivers (imported plants, direct flights and ferry connections) with risk factors related to establishment and spread (presence of potential insect vectors, vulnerable economic crops, alternative hosts and climate suitability) of this pathogen. This reveals that western European countries have the highest risk for entry, but that the Mediterranean basin runs the highest risk for establishment and spread of *Xf*. Lebanon in particular has the highest level of risk for *Xf* dispersal within its suitable territory. Countries without current outbreaks combining high risks of *Xf* arrival and establishment are mainly in the Mediterranean basin: Turkey is at the highest level of risk, followed by Greece, Morocco and Tunisia, which are ranked at the high level. The ranking model also confirms the vulnerability, in terms of invasion by *Xf*, of southern European countries (Italy, Portugal and Spain) in which the pathogen has already been reported. High summer temperatures in these southern countries are

likely to be the significant determinant for the overall invasion process, while northern European countries have a high level risk for the arrival of the pathogen, but relatively low summer temperatures may limit establishment and spread of major outbreaks. In general, our study provides a useful approach for mapping and comparing risks of invasive non-native species and emerging pathogens between countries, which could be useful for regional horizon scanning and phytosanitary and biosecurity management.

Keywords

alien species, biological invasion, entry, dispersal, plant bacterium, risk drivers

Introduction

Global trade networks are implicated in increasing rates of global spread of plant diseases through transport of live plants and other plant-related products (Chapman et al. 2017). The plant pathogenic bacterium *Xylella fastidiosa* (hereafter *Xf*) is an important example of this process. Originating in the Americas, this plant pathogen has recently been detected for the first time in several European and Near Eastern countries: Italy in 2013 (Saponari et al. 2013), France in 2015 (Denancé et al. 2017), Switzerland in 2015 (European and Mediterranean Plant Protection Organization 2015), Spain in 2016 (Olmo et al. 2017), Germany in 2016, Portugal in 2019, and more recently, Israel in June 2019 (European and Mediterranean Plant Protection Organization 2016, 2019a, b). This dangerous non-native pathogen causes several devastating plant diseases that have huge socio-economic and ecological impacts, motivating risk assessment for the entry and spread of *Xf* into new countries (European Food Safety Authority 2019).

Following detection of *Xf* outbreaks in Europe, the EU and some Middle East and North African (MENA) countries have implemented several risk reduction options to combat this plant disease and prevent its entry and spread. Despite these actions, there remains a risk that *Xf* will continue to spread to new countries and regions through the movement of infected host plants (asymptomatic or unknown hosts) or via unintentional transport of insect vectors through equipment as a commodity contaminant or vehicle “hitchhiker”. This transport risk is likely to be highest in countries with well-developed transport links to current outbreak areas (Chapman et al. 2017). However, transport connectivity will only present a serious risk of damaging outbreaks if there is also a suitable climate and land use to support the establishment and spread (Early et al. 2018). Therefore, there is a need for an overall country-level risk ranking for *Xf* simultaneously across the EU and MENA region which would allow the selection of priority countries for more detailed country-specific pest risk analyses.

As such, we aimed to rank the likelihood of potential invasion by *Xf* of new countries in these regions, and to provide an overall risk rating by combining rankings for entry, establishment and spread in order to assess each country’s overall vulnerability to *Xf* invasion. Over the past 20 years, there have been several model frameworks and studies of alien species risk assessment at an individual-country scale. These include invasive alien species risk assessment in Great Britain (Mumford et al. 2010), risk

assessment simultaneously prioritizing the control of invasive plant species and the conservation of rare plant species in the USA (Miller et al. 2010), risk assessment models for invasive species and uncertainty in rankings from multi-criteria analysis in Australia (Benke et al. 2010), a weed screening tool for the US (Koop et al. 2011), generic ecological impact assessments of alien species in Norway (Sandvik et al. 2013), risk screening tools for potentially invasive plants, animals and their pathogens in Belgium (D'hondt et al. 2015), and development of a plant risk evaluation tool to assess the invasive potential of ornamental plants (Conser et al. 2015). In contrast to these country-specific assessment, only a few studies assess risk across multiple countries, for example the EPPO prioritization process for invasive alien plants (Brunel et al. 2010), the new protocols for assessment of the environmental impact of pests in the EPPO decision support scheme for pest risk analysis (Kenis et al. 2012), guidance on environmental risk assessment of plant pests (European Food Safety Authority 2010), and the generic impact scoring system (Nentwig et al. 2016) in Europe. Moreover, a few protocols regarding alien taxa have been developed at the global level, such as the framework and guidelines for implementing the proposed IUCN (International Union for Conservation of Nature) environmental impact classification for alien taxa (Hawkins et al. 2015).

Concerning *Xf*, the few published studies that have performed risk assessment for this biological invader are limited to one country (Afechtal et al. 2018), one region (European Food Safety Authority, 2015, 2018, 2019; Godefroid et al. 2019;) or a subset of territories (Bosso et al. 2016) mainly based on climate suitability indicators. Thus, the novelty of the current research is to integrate multiple *Xf* risk drivers (i.e. plant trade, human movements, insect-vectors, host-plants, temperature suitability) and simultaneously ranks all countries in Europe and MENA region in a way that is useful for decision makers. In addition, these selected ranking variables (Table 1) are quantitative (except for vectors), objective (i.e. based on statistics and not on expert opinions), transparent, consistent, sufficiently specific, and available simultaneously to all the countries concerned. By quantifying these risk drivers with best available scientific evidence, the proposed approach is simple to apply and may be used as a conceptual framework in rating-based risk assessment of other pests to support policy for appropriate and efficient biosecurity management of *Xf* and other invading pests.

Methods

Countries included in the risk ranking model

Fifty six countries were selected for this study as shown in Figure 1. Thirty eight in Europe (Albania/ALB, Austria/AUT, Belgium/BEL, Bosnia Herzegovina/BIH, Bulgaria/BGR, Croatia/HRV, Cyprus/CYP, Czech Republic/CZE, Denmark/DNK, Estonia/EST, Finland/FIN, France/FRA, Germany/DEU, Greece/GRC, Hungary/HUN, Iceland/ISL, Ireland/IRL, Italy/ITA, Latvia/LVA, Lithuania/LTU, Luxembourg/LUX,

Macedonia/MKD, Malta/MLT, Moldova/MDA, Montenegro/MNE, Netherlands/NLD, Norway/NOR, Poland/POL, Portugal/PRT, Romania/ROU, Serbia/SRB, Slovakia/SVK, Slovenia/SVN, Spain/ESP, Sweden/SWE, Switzerland/CHE, Turkey/TUR and United Kingdom/GBR) and eighteen in MENA (Algeria/DZA, Bahrain/BHR, Egypt/EGY, Iraq/IRQ, Israel/ISR, Jordan/JOR, Kuwait/KWT, Lebanon/LBN, Libya/LBY, Morocco/MAR, Oman/OMN, Qatar/QAT, Saudi Arabia/, State of Palestine/PSE, Syria/SYR, Tunisia/YUN, United Arab Emirates/UAE and Yemen/YEM). Their selection was based on: (i) their connectivity through the trade of potentially infected plants for planting and the movement of people as passive vectors, (ii) their abundance of *Xf* host-plants, (iii) their relative summer climate similarity to countries where the disease is already present, and (iv) their inclusion in the EU and MENA regions.

Data on risk drivers for rankings

The vulnerability of a target country to the entry of *Xf* was assessed via six key risk drivers related to entry (importation of plants for planting, direct air flights and ferry traffic from countries source of *Xf*) and four risk drivers related to the establishment and spread of the bacterium (potential *Xf* vectors, vulnerable crops, alternative hosts from the forestry and ornamental sectors, and the suitability of summer temperatures) as summarized in Table 1. The use of these indicators was justified by: (i) the consideration of trade of plants and movement of passengers as common pathways of *Xf* interception mainly in Europe (European Food Safety Authority 2015), (ii) the biology and ecology cycle of the pathogen as stated by Chatterjee et al. (2008) (the presence of vectors is essential to transmit the bacterium from infected to healthy plants), (iii) the economic importance of *Xf* host-crops in southern European countries and MENA



Figure 1. Countries including in the risk ranking model, labelled with their international codes (ISO3).

Table 1. Risk drivers used to assess overall *Xylella fastidiosa* invasion risk and their weights.

Category	Code and description of the risk driver
Entry (plant trade)	<i>ENT</i> ₁ Live plant imports from three EU countries with major outbreaks (ESP, FRA, ITA).
	<i>ENT</i> ₂ Live plant imports from non-EU countries in which <i>Xf</i> is present but from which the bacterium has not been intercepted in Europe (ARG, CAN, IRN, PRY, TWN, and VEN).
	<i>ENT</i> ₃ Live plant imports from non-EU countries in which <i>Xf</i> is present and from which the bacterium has been intercepted in Europe (BRA, CRI, ECU, HND, MEX and USA).
Entry (human movement)	<i>ENT</i> ₄ Number of direct airline routes from or near outbreak regions in the EU (ESP, FRA and ITA).
	<i>ENT</i> ₅ Number of direct airline routes from non-EU countries in which <i>Xf</i> is present (ARG, BRA, CAN, CRI, ECU, HND, IRN, PRY, MEX, TWN, USA and VEN).
	<i>ENT</i> ₆ Number of annual ferry sailings from ports in or near outbreak regions in the EU (ESP, FRA and ITA) and from non-EU countries in which <i>Xf</i> is present (ARG, BRA, CAN, CRI, ECU, HND, IRN, PRY, MEX, TWN, USA and VEN).
Establishment and spread (vectors)	<i>EST</i> ₁ Presence of at least one known or potential <i>Xf</i> insect-vector.
Establishment and spread (host plants)	<i>EST</i> ₂ Proportion of agricultural area growing susceptible crops.
	<i>EST</i> ₃ Proportion covers of forest.
Establishment and spread (climate)	<i>EST</i> ₄ Mean relative <i>Xf</i> growth potential in vulnerable habitats, based on summer mean temperature.

region, (iv) the abundance of forest as *Xf* alternative hosts mainly in northern Europe countries (European Food Safety Authority 2018), (v) the summer optimal temperature (around 28 °C) for *Xf* growth as studied by Feil and Purcell (2001) and, (vi) their data availability to all selected countries.

As an indicator of the entry risk from plant trade we obtained data on import volumes of Plants for Planting from potential *Xf* source countries between 2000 and 2016 from the Resource Trade Earth database (<https://resourcetrade.earth/data>) and from the Taiwanese Bureau of Foreign Trade (<https://cus93.trade.gov.tw>) (Suppl. material 2: Table S1). *Xf* is known to infect over 563 cultivated and ornamental herbaceous, shrubby and woody species present in cultivated fields, gardens, parks, woods and forests could be infected by *Xf* through the effective transmission of insect vectors (European Food Safety Authority 2018). Many host-plants have long asymptomatic periods or remain fully asymptomatic, causing potentially high transport risk through trade. For each country, mean annual imports (metric tons) of plant commodity groups considered capable of carrying viable bacteria were obtained (i.e. bulbs, tubers, tuberous roots, corms and rhizomes in dormant and in growth; unrooted cuttings and slips; edible fruit trees, shrubs and bushes and live plants). Import volumes were calculated separately for three sets of countries in which *Xf* is present but potentially differ in import risk: (i) three EU countries with outbreaks (ESP, FRA, ITA), (ii) six non-EU countries from which the bacterium has not been intercepted coming into Europe (ARG, CAN, IRN, PRY, TW, VEN), and (iii) six non-EU countries from which *Xf* has been intercepted entering Europe (BRA, CRI, ECU, HND, MEX, USA) (European Food Safety Authority 2018 Annex D).

Intentional or unintentional human movement of contaminated plant material or insect-vectors may also lead to new introductions of *Xf*. To quantify the entry risk from human movement through international air travel, we calculated the number of annual

direct airline flights from the airports in or near to outbreaks areas in Europe (specifically demarcated zones in ESP, FRA and ITA) and from non-EU countries in which *Xf* is present (ARG, BRA, CAN, CRI, ECU, HND, IRN, PRY, MEX, TW, USA, VEN). Flight data were extracted from the OpenFlights database (<https://www.openflights.com>) which covers all flights in 2014. Entry risk from ferry traffic was evaluated using the number of annual passenger ferry sailings from ports in or near the outbreak areas in Europe and from the same infected non-EU countries. Ferry data were extracted from scheduled sailings in 2018 listed by Ferry Lines (<https://www.ferrylines.com>). We initially considered including road network connectivity as a risk factor, but decided against it because the relatively small outbreak areas in Europe are far by road from other uninfected countries.

Since *Xf* is entirely insect-transmitted, presence of potential vectors was considered an indicator of risk of establishment and spread. Disease transmission occurs by xylem-feeding insect vectors, mainly via spittlebugs in Europe. In the Apulia region of southern Italy, the spittlebug *Philaenus spumarius* (L.: Superfamily Cercopoidea, Family Aphrophoridae) is considered to play the major role in transmitting *Xf* subspecies *pauca* (Saponari et al. 2014). Additionally, other spittlebug species such as *Neophilaenus campestris* and *Philaenus italosignus* (Hemiptera: Aphrophoridae) are also able to transmit *Xf* (European Food Safety Authority 2015, 2018, 2019) suggesting that other unknown vectors could facilitate outbreaks if *Xf* were introduced to other countries. As such, for all countries, we searched for occurrence of the following potential xylem-feeding vector species: *Philaenus spumarius*, *Neophilaenus campestris*, *Aphrophora alni*, *Aphrophora salicina*, *Cercopis vulnerata*, *Cercopis sanguinolenta*, *Cicada orni*, *Cicadatra atra*, *Cicadivetta tibialis*, *Cicadella viridis*, *Lyristes plebejus* and *Tibicina haematodes*. Data were obtained from Global Biodiversity Information Facility (www.gbif.org), HemBases (<https://hemiptera-databases.org/>), 3I Interactive Keys and Taxonomic Databases (<http://dmtriev.speciesfile.org/>), Fauna Europea (<https://fauna-eu.org>) and a previous literature review (European Food Safety Authority 2015).

Establishment and spread also requires presence of *Xf* host plants. This risk indicator was estimated from the cultivated areas of its main vulnerable economic hosts (i.e. almonds with shell, apricots, blueberries, cherries, sour cherries, green coffee, citrus fruit nes, stone fruit stone nes, grapefruits including pomelos, grapes, olives, oranges, peaches and nectarines, pears, plums and sloes). Production data for 2000–2015 were obtained from the FAOSTAT database (<http://www.fao.org/faostat/en/>) and converted into the proportion of the total agricultural area of each country containing vulnerable crops. In addition, we obtained the proportion of the total area of each target country covered by forest, from the same source, as an indicator of alternative host plants for *Xf*, which is capable of infecting tree species from genera including *Quercus*, *Acer*, and *Ulmus*.

Risk from climate suitability was assessed based on summer land surface temperatures, obtained from two regional gridded layers deriving the mean temperature of the warmest quarter (Bio10) from MODIS satellite data. Europe was covered by the EuroLST layer at 250 m resolution from MOD11A1 V005 daily temperatures, re-projected to a 0.05 degree long-lat grid (Metz et al. 2014). Non-European countries were covered by the Tropical LST layer at 0.05 degree resolution from MOD11C3v5

monthly temperatures (Deblauwe et al. 2016). Before merging the layers, they were harmonized through a linear regression fitted to their overlapping areas ($\text{EuroLST} = 1.923 + 0.930 \times \text{Tropical LST}$, $R^2 = 0.983$). Summer land surface temperatures were converted into a relative *Xf* growth potential using published data on *in vitro* bacterial colony growth rates of *Xf* at different temperatures (Feil and Purcell 2001). From these data, a simple growth curve was fitted, with an optimum temperature of 28 °C and lower and upper growth temperatures of 8 and 35 °C (Suppl. material 1: Figure S1). This growth curve was rescaled between 0 and 1 and used to map the relative suitability of the summer land surface temperatures. Finally, mean suitable values within potentially invadable habitat (croplands and forests) were calculated for each country.

Risk ranking

We developed a structured system that ranks nations according to their risk of *Xf* invasion, combining the above risk drivers that constitute the components of the biological invasion process (Fig. 2). Biological invasion is a multistage process, but it can be simplified into two major stages: entry, involving the transportation and introduction of the species, and the subsequent establishment and spread of the alien species. Each stage involves a set of barriers or blocks that the species must overcome to successfully invade a new territory (Rogg et al. 2003; Blackburn et al. 2011; Seebens et al. 2015; Early et al. 2016). The overall invasion ranking from the entry and establishment and spread indicators mentioned above is based on their relative values among all countries (Early et al. 2016). An established matrix to combine both types of risk is used (Fig. 3).

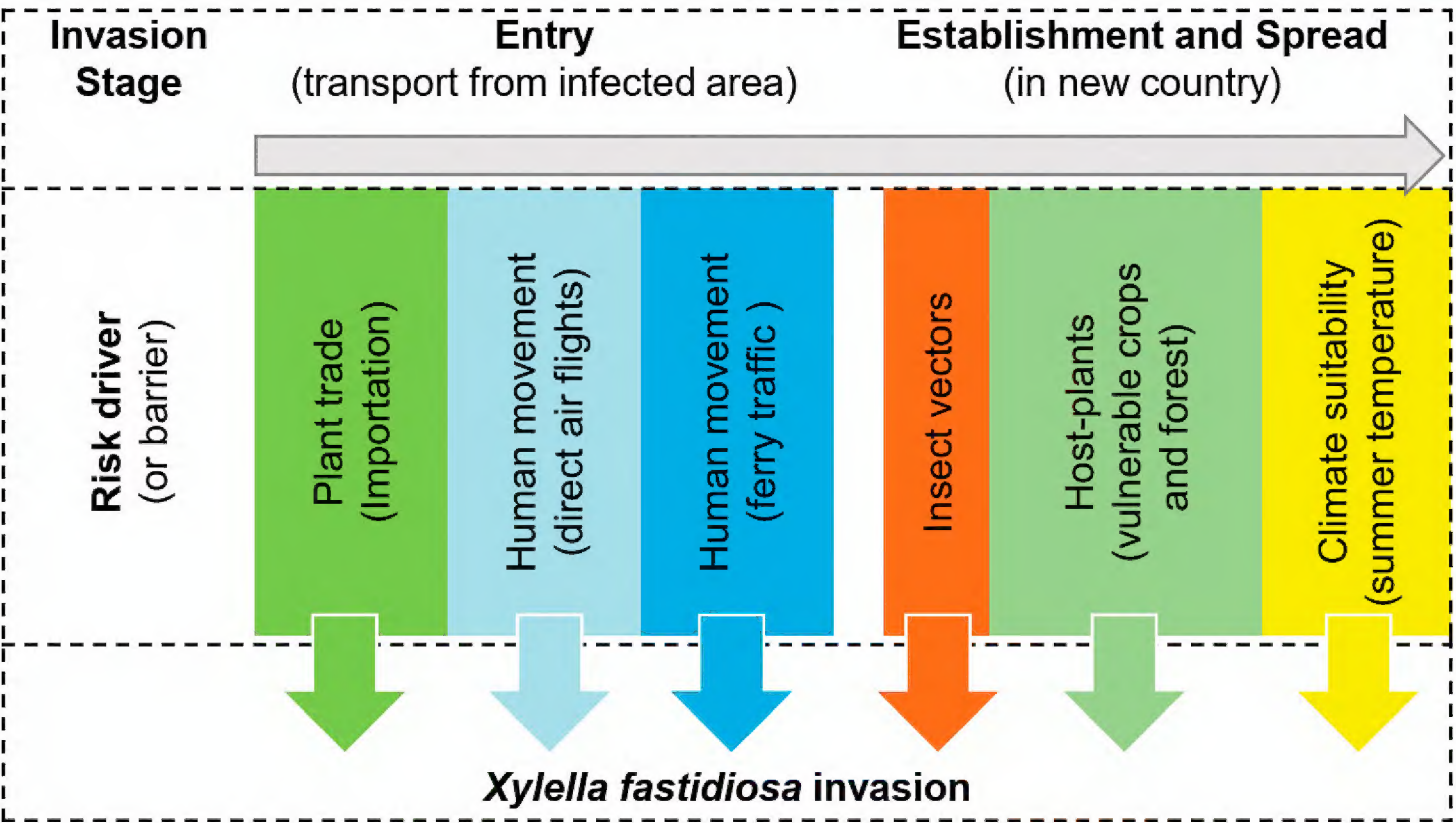


Figure 2. Overview of the invasion process of *Xylella fastidiosa* into a new country, based on the general framework of Blackburn et al. (2011). The diagram highlights specific risk indicators for each stage of invasion that were considered in our risk-ranking model.

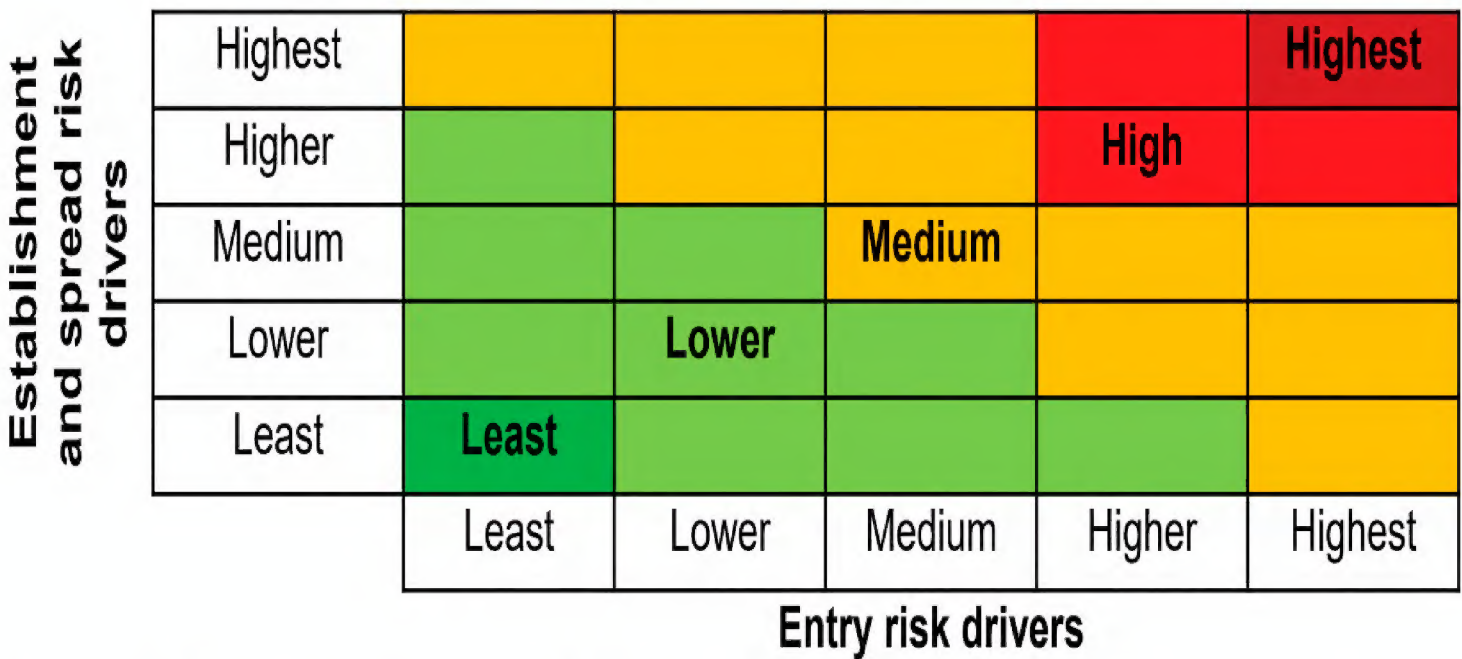


Figure 3. Martix for combining entry risk with establishment and spread risk to form an overall assessment of vulnerability to *Xf* invasion, based on Early et al. (2016). The diagram assembles five relative rank categories at each stage of invasion considered in our risk ranking model.

Variables in the analysis (risk driver data) were weighted based on their relative loadings or importance for the first two axes of a factor analysis (Table 2). Factor analysis has long been used as a multivariate method to combine correlated variables into smaller numbers of common factors (Venette 2015). In this study, it combined the multiple risk drivers into two dimensions of risk, which we interpreted as indicators of entry risk and establishment and spread risk. Prior to the factor analysis, data on all risk drivers were normalized by Box-Cox power transformation, improve their conformity to normal distributions. Bartlett’s test of sphericity was used to test for significant multivariate correlations between variables. In addition, the Kaiser-Meyer-Olkin statistic was used to describe the proportion of variance captured by the factor analysis. Inspections of the loadings showed that factors 1 and 2 correspond to increasing gradients of risk for entry, and establishment and spread, respectively.

Results

The risk indicators used in the ranking are shown in Figure 4. Factor analysis on these transformed indicators produced an adequate description of their multivariate pattern. Bartlett’s test of sphericity showed significant correlation among risk drivers ($\chi^2 = 305.2$, $df = 45$, $P < 0.001$), while the Kaiser-Meyer-Olkin statistic had a value of 0.695, which is above the 0.5 threshold that is usually accepted for adequate explained variation.

The loadings table (Table 2) shows high positive loadings for entry risk variables on the first factor, except for direct ferry lines (*ENT_d*), while none of the entry factors loaded strongly on the second factor. By contrast, establishment and spread risk drivers

Table 2. Rotated loadings of the risk drivers on two factor analysis axes, showing the contribution of each risk factor. See Table 1 for explanations of the risk driver codes. Relative loadings of entry drivers on factor 1 and of establishment and spread drivers on factor 2 were used as weights to estimate their combined risks.

Risk driver code	Factor 1 loading	Factor 2 loading	Entry risk weight	Establishment and spread risk weight
ENT_1	0.863	0.236	20%	
ENT_2	0.825	-0.258	19%	
ENT_3	0.815	-0.114	19%	
ENT_4	0.778	-0.074	18%	
ENT_5	0.750	-0.442	17%	
ENT_6	0.308	0.195	7%	
EST_1	0.366	0.703		25%
EST_2	-0.026	0.825		29%
EST_3	0.351	0.462		16%
EST_4	0.039	0.842		30%

loaded strongly on the second factor, except forest cover, but not on the first. Given this structure, the first factor was interpreted as a latent variable associated with entry, and the second factor interpreted as a latent variable indicating risk of establishment and spread.

Entry risk

Figure 5 displays the ranking of Europe and MENA countries according to the entry risk drivers. Most western European countries have high values of most entry risk indicators (Fig. 4) and as such have the highest overall entry risk. Most MENA countries appear less exposed to entry risk factors, except for Tunisia, Morocco, Jordan, Kuwait, Saudi Arabia and the UAE, where the entry risk level is high.

Establishment and spread risk

Figure 6 shows the ranking of Europe and MENA countries according to the four establishment and spread risk factors relating to insect vectors, host plants and climate suitability (see also Fig. 4). Among the establishment and spread risk indicators, suitable crops and summer temperatures were concentrated around the Mediterranean, leading to most Middle East and Mediterranean countries being classified at high to highest risk levels.

In the MENA region, Lebanon is the only country at the highest level of risk, followed by Morocco, Tunisia, Egypt, Israel and State of Palestine which are classified at high risk level. Algeria, Jordan and Syria are ranked at the medium level risk, while the remaining MENA countries are at least risk rank for Xf establishment and spread. In Europe, Albania, Cyprus, Greece, Italy, Macedonia, Malta, Portugal, Serbia, Spain and Turkey are classified at highest risk level, while Iceland, Ireland and Norway are at least risk level.

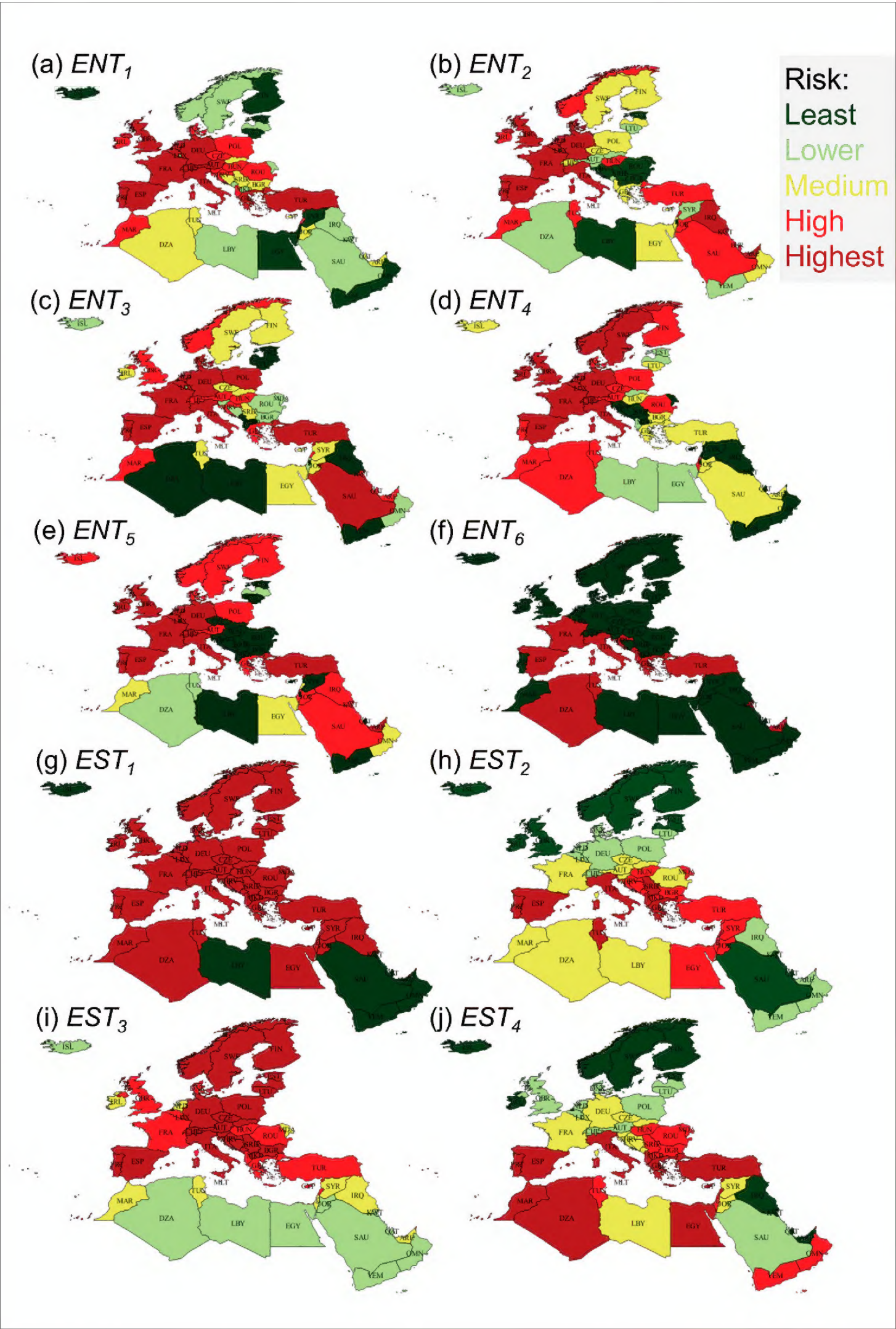


Figure 4. Maps of the risk indicators used for ranking potential for *Xf* entry (*ENT*) and establishment and spread (*EST*) as described in Table 1.

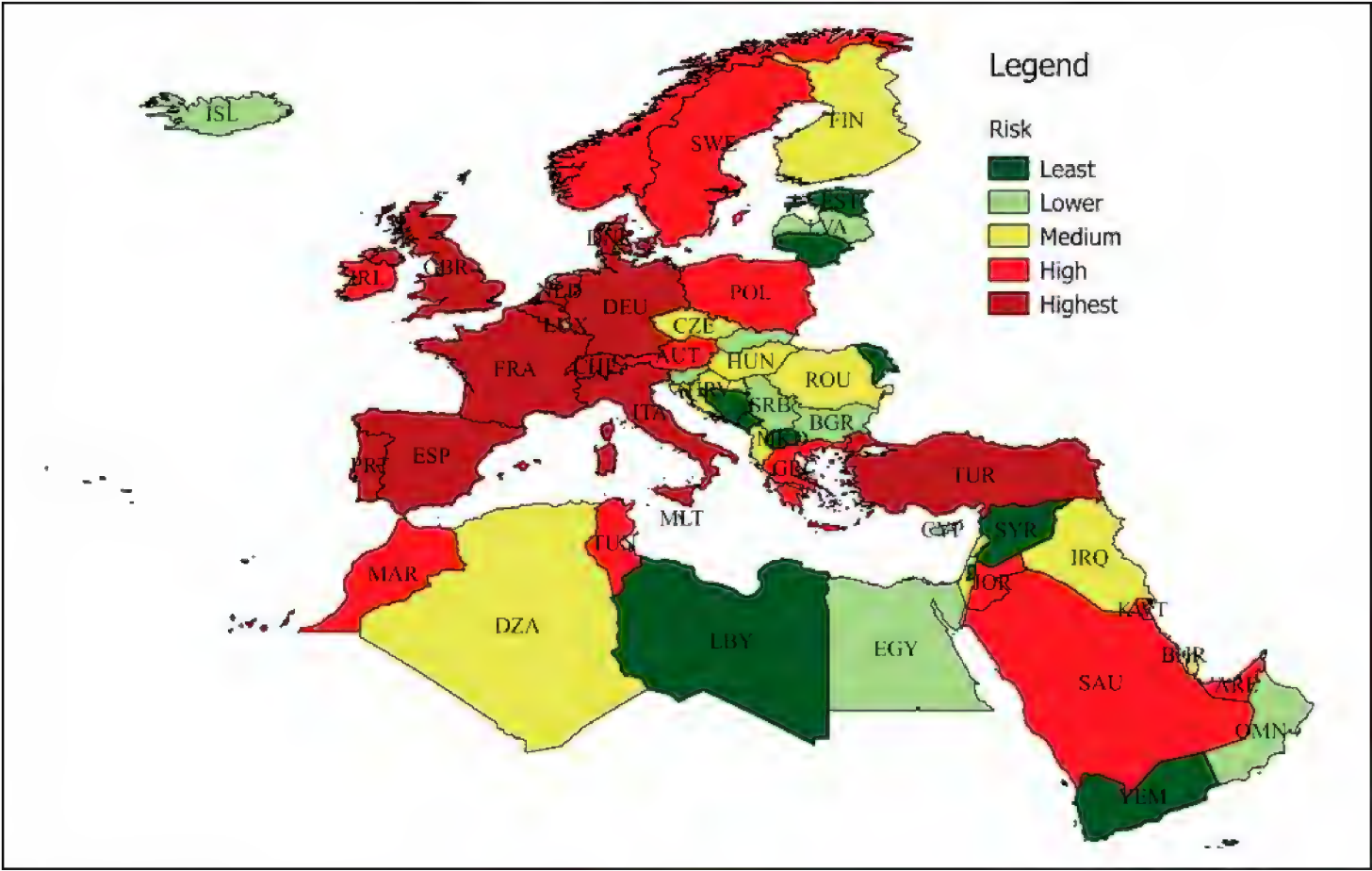


Figure 5. Rank categorization of Europe and MENA countries according to the six entry risk drivers of *Xylella fastidiosa* in relation to the importation of plants for planting, direct air flights and ferry traffic.

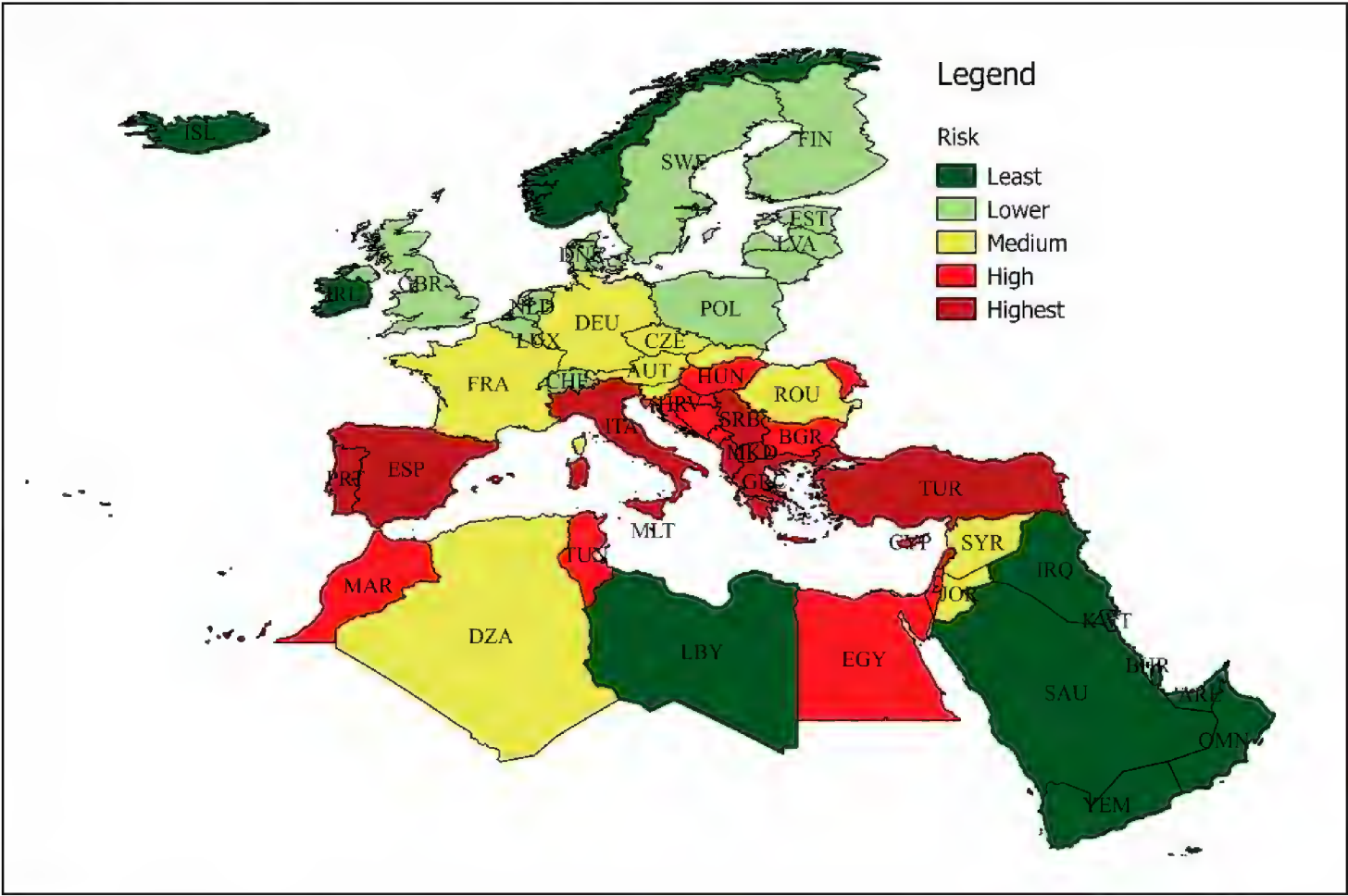


Figure 6. Rank categorization of Europe and MENA countries according to the four establishments and spread risk drivers of *Xylella fastidiosa* in relation to vectors, vulnerable economical crops, alternative hosts and climate suitability.

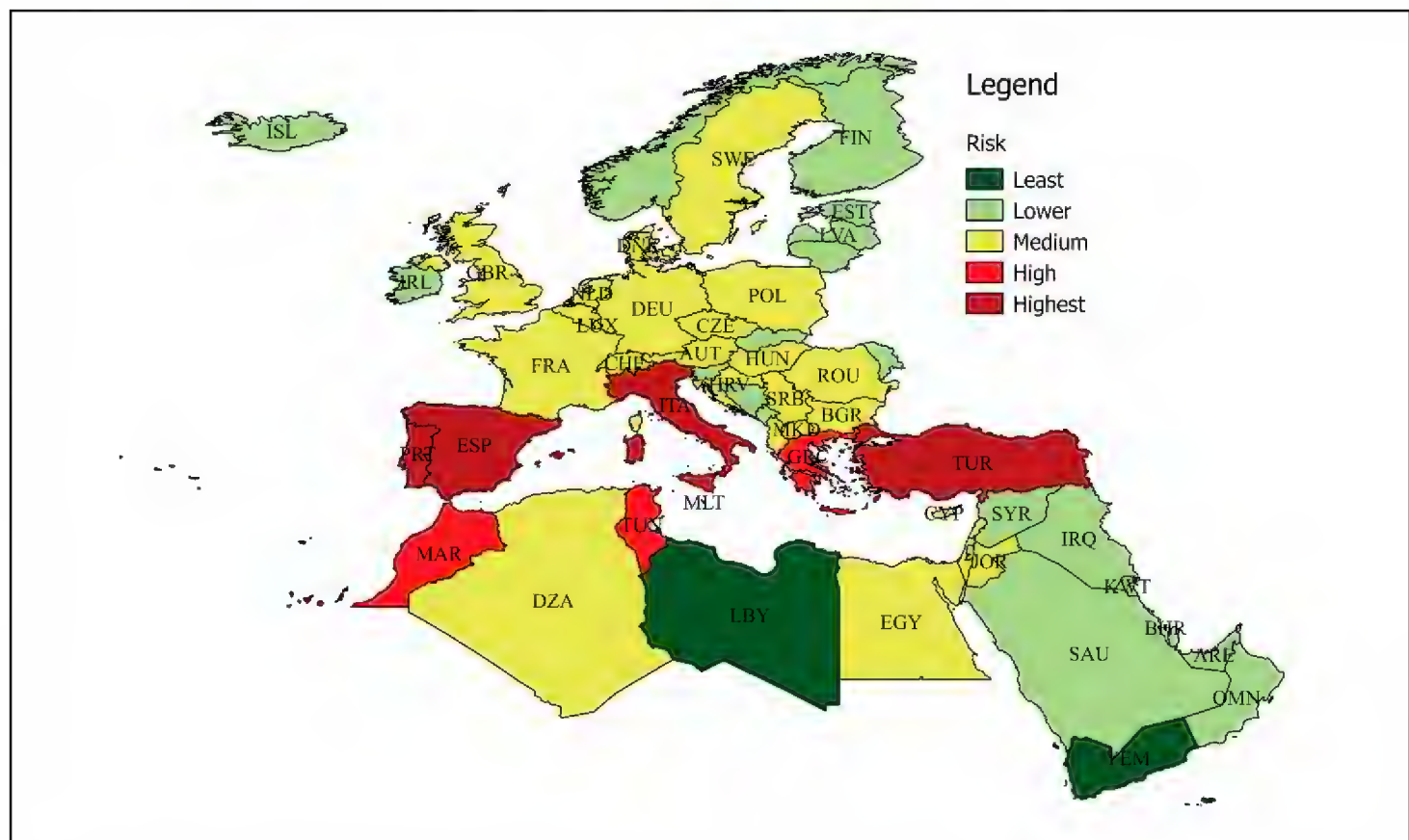


Figure 7. Overall rank categorization of Europe and MENA region according to exposure to invasion by *Xf* with respect to combined all entry, establishment and spread risk drivers and using the matrix of Early et al. (2016).

Overall risk of exposure to invasion by *Xf*

When we combined the risk rankings for entry (Fig. 5) and establishment and spread (Fig. 6) using the matrix in Figure 2 the countries ranked as most vulnerable were Italy, Portugal, Spain and Turkey (Fig. 7). Additionally Greece, Morocco and Tunisia were ranked at a high risk level. Only three countries (Bahrain, Libya and Yemen) yield the least level of combined risk, although the risk was generally low in northern Europe and the non-Mediterranean Middle East.

Discussion

The threat of intentional or unintentional species movements leading to the entry and spread of invasive alien organisms is increased by international trade and travel (Tatem 2009; Early et al 2016; Chapman et al 2017). For *Xf*, recent outbreaks and interceptions of infected imported plants for planting in Europe show that *Xf* spreads to new countries via this pathway (European Food Safety Authority 2015). Most countries in Europe and the MENA region are increasingly connected with each other and with global sources of *Xf* in the Americas and Asia through the trade in imported plants and human travel. Given weaknesses in current phytosanitary regulations and airport inspection structures in parts of the region, especially in some MENA countries, it is possible that passengers could carry with them potentially infected planting material, or

that transport infrastructure could accidentally carry *Xf* insect vectors. Consequently, there is a real risk that *Xf* may continue to overcome geographical barriers and infect new countries, potentially leading to major new disease outbreaks. Our assessment of the joint risks of *Xf* entry and establishment and spread therefore provides a useful approach for regional risk assessment by ranking the relative risk across 56 countries.

In particular our analysis identified a contrast between entry risk and the risk of establishment and spread. This was clearly seen in the factor analysis loadings, in which entry risk drivers loaded strongly on axis 1, while establishment and spread risk drivers loaded strongly on axis 2. Based on this, countries in western Europe and also Turkey tended to score highly for entry risk, principally because they import greater volumes of plants from infected countries in Europe and globally and had greater numbers of direct flight connections originating in infected regions. By contrast, risk of establishment and spread was ranked mostly based on the degree to which crops grown in a country are known to be susceptible to *Xf* and whether the summer temperature was apparently well suited to *Xf* colony growth. Presence of vectors was also weighted strongly in the factor analysis, but did not have a big influence on the results since nearly all countries had at least one potential vector species recorded as present (Fig. 4g). Countries with vulnerable crop types and summer mean land surface temperatures close to the optimum of 28 °C (Feil and Purcell 2001) were mainly located around the Mediterranean basin, where many countries were assessed to have low entry risk. The known world distribution of *Xf* is shown in the suppl. material 5: Table S4.

Consequently, few countries were ranked very highly for both entry risk and establishment. Of those that were, three countries already have major *Xf* outbreaks, namely Italy, Spain and Portugal, whose outbreak was discovered after this study was conducted. Greece and Turkey were also ranked in the highest risk group but so far remain disease free. Güldür et al. (2005) reported the detection of *Xf* on seven almond trees uprooted in Turkey. However, following this detection, the Turkish National Plant Protection Organization declared that *Xf* did not occur in the country. Outside of the top-ranked countries, Morocco and Tunisia were ranked as high risk countries, on the basis that they scored fairly highly for both entry and establishment and spread. So far, *Xf* has not been detected in those countries. However, a pest risk analysis conducted for Morocco concluded that the overall likelihood of *Xf* spread in the country is very likely, providing support for our assessment (Afechtal et al. 2018). Interestingly, in our study France was ranked as medium overall risk, despite it having outbreaks on its south coast and on the Mediterranean island of Corsica. The primary reason for this is that France also includes more northerly regions with less suitable summer temperatures and less vulnerable crop types. Therefore, despite France clearly having areas at high risk of the disease, the national-scale resolution of the study reduced its overall risk ranking.

Our findings are broadly consistent with other risk assessment studies for *Xf* in Europe and MENA region. For example, data-driven species distribution modelling studies using a wider range of climate variables than we assessed generally confirm our simple mapping of temperature risk (Bosso et al. 2016; European Food Safety Authority 2019). For example, Godefroid et al. (2019) predicted the potential distribution of *Xf* subsp. *fastidiosa* in Spain, France, Italy, Croatia, Greece and Turkey, and the coastal

regions of North Africa, which is consistent with our simple forward projections based only on an *in vitro* growth curve (see Suppl. material 1: Fig. S1). In addition there have been recent detections of *Xf* in some of the countries that we rated as high risk for establishment based on temperature and crop types. This includes Lebanon, in which Temsah et al. (2015) reported *Xf* on oleander, although Habib et al. (2016) disagreed with this finding. It also includes Israel, in which *Xf* is under containment after a recent detection on almonds in the Hulla Valley (European and Mediterranean Plant Protection Organization 2019b). By contrast, countries such as Germany, Switzerland and Netherlands were ranked as having low or medium risk for establishment and spread. In those countries *Xf* has been intercepted in trade or infected plants within protected indoor locations (consistent with their highest ranking for entry), but has not been able to spread to the wider environment (European Food Safety Authority 2019).

Limitations of the study

Important limitations of this study include its country-level resolution, reliance on data of differing quality, missing risk factors with insufficient data to include and uncertainty about how to combine risk factors into overall risks. As discussed above, the country-level resolution of the analysis affected results for countries that appear largely unsuitable for *Xf*, but have small areas that are at high risk, such as France. Future approaches could use high resolution gridded data on the risk drivers to try to map risk at a higher resolution, addressing this problem. One reason that we were limited to a country-level analysis was that some datasets were only available at that resolution. Notably international trade data is only available for countries and vector distributions are too poorly mapped to allow regional breakdowns. As plant trade is the major pathways of *Xf* introduction, there seems little prospect of mapping entry risk at higher resolutions. However, availability of gridded climate and land use data (see Suppl. material 1: Fig. S1) could allow higher resolution mapping of those components of establishment and spread risk.

Due to lack of adequate data across Europe and the MENA region we did not feel able to include some other potentially relevant risk drivers. Individual host plant species (other than major crops) and insect vector distributions were not mapped well enough to consider. We also did not consider variation in risk for different subspecies of *Xf*, of which at least three are present in Europe and the MENA (*Xf* subsp. *pauca*, *multiplex* and *fastidiosa*) and all differ in host plant range and temperature-growth responses (European Food Safety Authority 2019). In addition, lack of information meant our the analysis did not account for variation in management regimes in different countries, including farmers' cultural management (i.e. crop genetics, use of resistance/tolerant cultivars, presence of transgenic plants, vegetation, vector control etc.), surveillance and monitoring programs, or phytosanitary regulations at the import stage or testing capacity. However, with more complete data the present ranking model could be extended to include additional risk indicators.

The results of this type of study can also be sensitive to how risk indicators are combined. We suggest that our use of factor analysis to weight additive risk combina-

tion ensured that our individual risk rankings for entry and establishment and spread followed the major gradients in the assessed drivers of those risks. In addition, we explored alternative schemes, including multiplicative risk combinations, and found these produced qualitatively similar results. In addition, we used an established matrix to combine both types of risk (Fig. 3; Early et al 2016), though it is likely that our results are somewhat sensitive to this choice of matrix. Furthermore, relative risk rankings such as those produced here are sensitive to the set of countries included in the analysis, which is why we endeavored to include the widest range of countries in the geographical regions that may be threatened by *Xf*'s arrival in Europe. Ideally, we would convert our qualitative rankings into quantitative probabilities of entry and quantitative measures of spread potential. However, until large numbers of entry and outbreak events are observed, allowing us to link particular risk drivers to actual probabilities of invasion it will be difficult to improve upon qualitative ranking.

Conclusion

Regional risk assessment for high-impact invasive alien species such as *Xf* requires approaches that incorporate multiple risk drivers to simultaneously rank countries for multiple stages of invasion, such as the approach developed here. The world is increasingly connected by international plant trade and human travel, which are potential drivers of *Xf* entry into new areas where the presence of insect vectors, the abundance of host-plants as well as the climate suitability play an important role for its dispersal. As such our approach could be useful for both individual countries to understand their risk of *Xf* relative to other countries, and if applied across many different pests it could be useful to identify priority species. It is also useful for supra-national organizations interested in Plant Health (i.e. EPPO, EFSA, and EU) who can use country-level risk rankings to prioritize phytosanitary resources among countries. In this context, the strength of this study is that it creates a tool for mapping, ranking and combining multiple sources of invasion risk at country-level.

Overall, we identified the most vulnerable new countries to *Xf* invasion are mainly located in the Mediterranean basin, particularly Turkey, Greece, Morocco and Tunisia. As such, this research provides important information in terms of potential exposure by *Xf*, for policy makers or stakeholders in high risk countries where *Xf* has not yet been reported. We suggest that these countries and other ranked with relatively high risk should conduct detailed individual risk analysis, take preventive measures, and if necessary, improve their surveillance systems for early *Xf* detection in plants and insect-vectors, and raise awareness to prevent socio-economic and ecological impacts on their ecosystems. In addition, our approach could be adapted to assess the specific risks for other important invasive alien species, irrespective of their origin, potential area of invasion and whether or not they have already invaded parts of the risk assessment region. As such, it provides a useful addition to tools and methods more commonly applied in regional-scale risk assessment for invasive alien species.

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Supplementary material 1

Figures S1, S2

Authors: Michel Frem, Daniel Chapman, Vincenzo Fucilli, Elia Choueiri, Maroun El Moujabber, Pierfederico La Notte, Franco Nigro

Data type: measurement

Explanation note: To map gradients of *Xylella fastidiosa* relative climate suitability in Europe and Middle East and North Africa countries.

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Link: <https://doi.org/10.3897/neobiota.59.53208.suppl1>

Supplementary material 2

Table S1

Authors: Michel Frem, Daniel Chapman, Vincenzo Fucilli, Elia Choueiri, Maroun El Moujabber, Pierfederico La Notte, Franco Nigro

Data type: measurement

Explanation note: Importation of plants for planting from countries, source of *Xylella fastidiosa*.

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Link: <https://doi.org/10.3897/neobiota.59.53208.suppl2>

Supplementary material 3

Table S2

Authors: Michel Frem, Daniel Chapman, Vincenzo Fucilli, Elia Choueiri, Maroun El Moujabber, Pierfederico La Notte, Franco Nigro

Data type: measurement

Explanation note: Correlation matrix of the ranking model.

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Supplementary material 4

Table S3

Authors: Michel Frem, Daniel Chapman, Vincenzo Fucilli, Elia Choueiri, Maroun El Moujabber, Pierfederico La Notte, Franco Nigro

Data type: measurement

Explanation note: Rank categorization of European and Middle East and North Africa countries according to exposure to invasion by *Xylella fastidiosa* and classified from the highest to the least overall risk rank.

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Link: <https://doi.org/10.3897/neobiota.59.53208.suppl4>

Supplementary material 5

Table S4

Authors: Michel Frem, Daniel Chapman, Vincenzo Fucilli, Elia Choueiri, Maroun El Moujabber, Pierfederico La Notte, Franco Nigro

Data type: occurrence

Explanation note: The known world distribution of *Xylella fastidiosa*.

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Link: <https://doi.org/10.3897/neobiota.59.53208.suppl5>